of the levels from normal littermates. The anemia phenotype improves as the animals age and, by three weeks, the *Xla* females are virtually indistinguishable from normal littermates. This suggests that there is a defect in a developmental shift in hematopoiesis and RBC production in *Xla* females. The *Xla* gene maps to a small region near the centromere at the top of the X chromosome near the *Gata-1* gene. The *Xla* locus is 3 cM proximal to the CA dinucleotide repeat genetic marker *D17Mit163* and shows no recombination with the *Gata-1* gene. However, no mutation has been found in the *Gata-1* gene by sequencing. The *Xla* mutation is severe in that males carrying one copy of the *Xla* gene are never born whereas *Xla*/+ females with one copy of the mutant gene do survive into adulthood. A large backcross has been established for high resolution mapping of the *Xla* gene. We are in the process of identifying candidate genes for the *Xla* mutant.

**Abstract# 1847** **Poster Board #-Session: 18-II**

**Diamond-Blackfan Anemia (DBA): Identification of Nonpenetrant Individuals in the Molecular Era.** Blanche P. Alter,[1] Lisa Leathwood*,[2] June Peters*,[1] Ann Carr*,[2] Dale Singer*,[3] Kirk Aleck*,[3] Lori Wagner*,[3] Mark H. Greene*.[1] *[1]Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA; [2]Westat, Rockville, MD, USA; [3]Department of Pediatrics, Phoenix Children's Hospital, University of Arizona, Phoenix, AZ, USA.*

To determine the penetrance of DBA in a large kindred with previously described dominantly inherited DBA, we analyzed the integrity of the RPS19 gene. We examined 30 persons representing 3 generations, of whom 22 were in the DBA lineage and 8 were unrelated spouses. Group and individual genetic education and counseling sessions were provided. Studies included a brief history and physical examination, clinical photographs of face and hands, a complete blood count, red cell adenosine deaminase (ADA) and other enzymes, fetal hemoglobin (Hb F), and sequencing of the RPS19 gene. All laboratory studies were performed in CLIA-certified facilities. Data were adjusted for age, sex, and altitude. Eight participants were thought to have DBA based on prior clinical laboratory results, and 22 were thought not to have DBA. All 8 thought to have DBA had high MCV and red cell ADA, 6 had increased Hb F, short stature, and hypoplastic thenar eminence, and 4 had low Hb. Among those who were not thought to have DBA, 5 had increased MCV, 0 had increased ADA, 1 had increased Hb F, 1 was short, 3 had hypoplastic thenar eminence, and 2 had low Hb. Only ADA and MCV were 100% sensitive, and only ADA was 100% specific. The proband's RPS19 gene was fully sequenced, and a heterozygous mutation (G185A) was found in exon 4. All other DNA samples were then examined for this mutation, and the 8 known DBA patients were confirmed. However, one sample from an individual thought not to have DBA was unexpectedly found to have the RPS19 mutation: an 11 year old female, with Hb 12.7 g/dl, MCV 89.4 fl (normal 77 - 92), Hb F 0.8%, height >50%ile, and normal thenar eminence. ADA was 0.95 EU/gm Hb (upper limit 0.96), and enolase was 3.1 EU/gm Hb (upper limit 3.1). One man who had been told he did not have DBA but was "a carrier", had normal laboratory and physical findings, and was wild type for RPS19. Thus RPS19 mutation results differed from the clinical impressions, and reclassified 2 family members in this kindred. The nonpenetrant individual had a truly silent phenotype, and only RPS19 mutation testing (in 3 independent DNA samples) permitted correct classification. Diagnosis of individuals with DBA mutations without signs or symptoms is critical since they may be proposed as hematopoietic stem cell donors for siblings with severe DBA. The clinically asymptomatic individuals may be at increased risk of leukemia, solid tumors, or other complications which have been associated with classical DBA. Only long term follow-up of DBA-mutation-positive, clinically nonpenetrant individuals will provide information regarding the phenotypic spectrum of individuals with DBA.

**Abstract# 1848** **Poster Board #-Session: 19-II**

**Paroxysmal Nocturnal Hemoglobinuria (PNH): Identification of a Highly Polymorphic Gene Expressed by PIG-A Mutant Cells.** Gabrielle Meyers,[1] Teresa M. Vreeke*,[2] Charles J. Parker.[1,2] *[1]Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA; [2]Medicine, VA Medical Center, Salt Lake City, UT, USA.*

Mutations in PIG-A account for the deficiency of glycosyl phosphatidylinositol-anchored proteins (GPI-AP) on the hematopoietic elements of PNH, but the basis of the clonal expansion of the mutant cells is enigmatic. Further, individual patients frequently have multiple clones with discrete PIG-A mutations. The in vivo proliferative (or survival) characteristics of these PIG-A mutant clones, however, are not uniform as their individual contribution to hematopoiesis varies greatly. Together, these observations suggest that factors independent of the PIG-A mutation contribute to clonal expansion and clonal dominance and that there is heterogeneity in the proliferative/survival characteristics of the mutant clones. The purpose of these studies was to identify difference in gene expression that account for the heterogeneous characteristics of PIG-A mutant cells in PNH. We previously studied a patient with PNH who was found to have four discrete PIG-A mutations (Blood 87:2546-2557, 1996). One of the mutant stems cells accounted for 75% of total hematopoiesis, while combined, the other 3 clones accounted for 20%. In the present studies, the lymphocytes from that patient were treated with aerolysin to remove cells with normal expression of GPI-AP. Subsequently, the residual PIG-A mutant, GPI-AP deficient lymphocytes were expanded in culture. Representational difference analysis (RDA) of cDNA was used to compare gene expression by these cells with that of a pool of cultured lymphocytes from normal donors. RDA is a form of subtractive hybridization is which one cDNA population (the driver) is hybridized in excess against a second population (the tester) to remove common sequences, thereby enriching for sequences unique to the tester. In the present experiments, cDNA from the PIG-A mutant cells was used as tester with cDNA from the normal donor pool used as the driver. After 3 rounds of hybridization/subtraction (the last at a driver:tester ratio of 400,000:1), the difference products were cloned. Forty clones were analyzed by nucleotide sequencing, and 15 discrete sequences were obtained. Only 3 of the 15 unique sequences were derived from known genes (GST-theta, acyloxyacyl hydrolase, and interferon regulatory factor 5). Of the 12 other difference products, two were identified as head to tail restriction fragments of the same linear sequence of cDNA and were chosen for further analysis. The length of the combined difference products was 823 bp, and the composite difference product was called 9+3. Northern analysis showed an intense band (above the 28S ribosomal marker) that was present in the RNA from the PNH cells but absent or of lesser intensity in the control RNA. Southern analysis was consistent with both quantitative and qualitative differences in the Bam H1 and Bgl II restriction patterns when the blots were probed with oligonucleotides derived from 9+3. These findings suggest that the gene that encodes 9+3 is present in multiple copies or that there are other homologous regions within the genome (or both). Both PCR and RT-PCR products generated by primers designed to amplify regions of 9+3 were sequenced. This analysis showed remarkable heterogeneity. Many nucleotide differences were observed and a dinucleotide microsatellite within 9+3 varied by 2-8 nucleotides. These studies have identified a novel gene that is expressed by PIG-A mutant cells. Further analysis of this highly polymorphic gene may provide insight into the basis of the heterogeneous growth/survival characteristics among PIG-A mutant hematopoietic stem cells that is characteristic of PNH.

**Abstract# 1849** **Poster Board #-Session: 20-II**

**K562 Cells Transfected with Mutant Ribosomal Protein S19 (RPS19) Have a Limited Protein Translation Rate Suggesting a Dominant Negative Effect of RPS19 Mutations in Diamond-Blackfan Anemia.** Jana Cmejlova*,[1] Filip Vrbacky*,[1] Ota Fuchs*,[1] Dagmar Pospisilova*,[2] Radek Cmejla*,[1] (Intr. by Jarosiav Jelinek) *[1]Dpt. of Cell Physiology, Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [2]Dpt. of Pediatrics, Palacky University, Olomouc, Czech Republic.*

Diamond-Blackfan anemia (DBA) is a rare congenital disorder characterized by normocellular bone marrow with a selective deficiency in red cell precursors, and macrocytic normochromic anemia presenting usually in the first year of life. The only gene connected with DBA is ribosomal protein S19 (RPS19), it has been found mutated in 25% of patients. However, the exact relationship between RPS19 mutations and DBA pathogenesis remains unclear. Two possible mechanisms can be proposed: a defective function of RPS19 in translation, and/or a disruption of an extraribosomal function of RPS19. We focused on the role of RPS19 in protein translation and its connection to cell proliferation. We transduced the K562 erythroleukemic cell line with vectors expressing either wild type (wt) RPS19 cDNA or one of four mutant RPS19 cDNA variants previously identified in our DBA patients (R56Q; R62Q; 11 bp deletion leading to the frameshift at codon 66 and stop at codon 149; 8 bp insertion leading to the frameshift at codon 131 and no stop codon). A significant decrease in the rate of translation was observed in all K562 transfectants expressing mutant versions of RPS19 except for the 11 bp deletion. This deletion causes changes in the amino acid sequence so gross that the resulting protein is unlikely to participate in the process of translation (only 66 aa from 145 aa are preserved). In spite of the decrease in protein translation rate, no significant differences were detected in the proliferation rate of the K562 cells transfected with wt or mutant RPS19 cDNAs. Our ongoing studies focus on the ability of individual mutants of RPS19 to constitute the small ribosomal subunit and on the measurements of translation rate in cells from DBA patients. We conclude the rate of translation was significantly lowered in K562 cells transfected with cDNA of mutant RPS19 species (except for the 11 bp deletion). This decrease in translation rate was clearly manifested, although the K562 cell line has three intact copies of RPS19. We suggest that limited protein synthesis due to a dominant negative effect of mutant RPS19 on translation may be the primary cause of DBA phenotype in patients carrying RPS19 mutations.

**Abstract# 1850** **Poster Board #-Session: 21-II**

***Hprt* "Mutations" in Paroxysmal Nocturnal Hemoglobinuria (PNH) Reflect T-Cell Clonal Expansion, Not Genomic Instability.** Guibin Chen, Weihua Zeng*, Spencer Green*, Neal S. Young. *Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.*

In paroxysmal nocturnal hemoglobinuria (PNH), acquired mutations in the PIG-A gene of an hematopoietic stem cell leads to defective biosynthesis of glycosylphosphatidylinositol (GPI) anchors, failure to express GPI-anchored proteins on the surface of the cell's progeny, and clinical intravascular hemolysis, venous thrombosis, and defective hematopoiesis. Disease-causing mutations are located randomly in the PIG-A genetic sequence, without hot spots, and multiple genetically defined PNH clones may coexist in a single patient. PNH is associated with myelodysplasia and cytogenetic abnormalities. Genomic instability has been postulated to underlie these laboratory and clinical findings. Mutation analysis of the *hypoxanthine-guanine phosphoribosyltransferase* (*hprt*) gene has appeared to support this hypothesis in some (Horikawa K, et al, Blood 2002;99:24) but not other (Purow BD, et al, Blood Cells Mol Dis 1999;25:81) studies. *Hprt* mutations block the purine salvage pathway, producing resistance to purine analogs; clonal selection in 6-thioguanine (6-TG)-containing medium has provided a simple laboratory assay of mutation frequency. However, because lymphocytes are most abundant and easily grown from peripheral blood, an apparent increase in *hprt*- cells may be the result either of a true increase in the mutation frequency or of clonal T cell expansion. In order to resolve these possibilities, we analyzed the molecular and functional features of *hprt* mutants in peripheral blood mononuclear cells (PBMNCs) from eight PNH patients. CD8 cells predominated over CD4 cells in these samples (41.8% ± 4.9 versus 35.5% ± 8.4), as measured by flow cytometry; approximately half of CD8 lymphocytes (47.5% ± 12.2) lacked GPI-anchored protein expression (CD55 and CD59 combined as markers), but only a small proportion of CD4 cells (5.7% ± 4.3) appeared to derive from the PNH clone. Using a conventional negative selection strategy of cell culture in the presence of 6-TG, the *hprt* mutant frequency (Mf) in T lymphocytes in PNH was $3.25 \times 10^5 \pm 1.03$, significantly higher than in normal controls, $2.81 \times 10^6 \pm 1.25$ ($P<0.05$). (We were unable to assess *hprt*